JOTTINGS FROM THE BIOLOGICAL LABORATORY OF SYDNEY UNIVERSITY.

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8. Notes on Tmesipteris and Psilotum.

These two remarkable genera are made by Goebel in his "Outlines of Classification and Special Morphology of Plants" (English translation, 1887, p. 282), to constitute a separate division, the *Psilotaceæ*, of the *Lycopodinæ*. The two genera, though differing a good deal superficially, are yet in essential points nearly allied, and quite distinctly separated off from the true Club-Mosses.

Thesipteris tannensis, Bernh., is found growing most commonly on the stems of species of tree-fern (Alsophila and Dicksonia) sometimes on the ground, in New South Wales and Tasmania. It occurs also in Queensland, Victoria, New Zealand, and the Pacific Islands. The following is the definition of the genus given by Bentham and Mueller in the "Flora Australiensis."*

"Stems simple, leafy. Leaves vertical, sessile and decurrent, entire, intermixed with leafy bracts bipartite on a short petiole. Spore-cases usually two together, united into a capsule-like sorus, sessile on the petiole of the bracts, transversely oblong, flattened, two-celled and didymous or 2-lobed, opening loculicidally in two valves. Spores minute, uniform."

And the following is the description given of the species:-

"Stems from a creeping slender rootstock ascending or pendulous, 6 inches to 1 foot long. Leaves obliquely oblong or narrow lanceolate, usually about $\frac{1}{2}$ inch long, but sometimes nearly 1 inch, truncate obtuse or acute at the end, the lower margin shortly decurrent, the single central nerve often produced at the end into a fine point. Bracts rather shorter than the leaves and occasionally replacing them in the upper part of the stem, deeply divided into two segments like the leaves but smaller and more acute. Capsule-like sori about 2 lines broad and 1 line long, much compressed, parallel to the petiole."

Tmesipteris presents a creeping rhizome and a series of leafbearing stems. In nearly all the specimens I have seen the rhizome is found deeply buried in the fibrous coating of the stem of the Tree-fern, while the leaf-bearing axes hang downwards; more rarely the plant is found creeping on the surface of the ground; it branches dichotomously and may attain a length of several feet. None of my specimens have any roots. The branches either end in rounded extremities, or are continued into aerial leaf-bearing stems. The rhizome is cylindrical, finely ridged in a longitudinal direction, and covered with short scattered brown hairs. It contains a central bundle of small scalariform and reticulated vessels with thin-walled elongated cells (and sieve tubes?) without any definite sheath, though the cells immediately surrounding the bundle take on a special appearance in many parts of the rhizome owing to their containing a dark brown matter. The surrounding tissue consists of thin-walled cells elongated in the direction of the long axis of the rhizome, and containing, except near the growing point, only a small quantity of protoplasm. The epidermis is not strongly thickened; it bears here and there blunt projections forming the bases of the hairs.

The leaf-bearing stems or aerial branches are cylindrical at the base, but further up they are marked by the longitudinal ridges continuous with the decurrent leaves. The cells of the epidermis

are elongated in the direction of the long axis of the stem; the outer wall, in the leaf-bearing part of the stem, but not in the basal part, is irregularly thickened, leaving rounded or slit-like depressions. The cortical tissue is strongly sclerenchymatous in its outer part, with abundant cell-contents, and with numerous intercellular spaces. Internally the walls of the cells are thinner and the cell contents scanty, so that this part of the cortex may be regarded as representing the endodermis. Surrounding the central vascular bundle is a ring of a dark brown homogeneous substance, which is so arranged that it forms continuous branching and anastomosing longitudinal lines breaking through apparently from cell to cell, and thus constitutes a network enclosing the bundle. This brown layer is present in nearly all parts of the stems both creeping and aerial; it varies in thickness and may sometimes be found to be entirely absent for a short space. When at its thickest it occupies about four layers of cells; near the growing point of the underground stem it sometimes breaks through into the interior of the vascular bundle, and fills the interior of some of the spiral vessels. This brown matter is solid, and is quite insoluble in water, cold or boiling, in boiling absolute alcohol, ether, chloroform, turpentine, and liquor potassiæ; a similar substance occurs in Psilotum, as will be noticed below. Internal to the brown layer there is nearly always a single layer of thin-walled cells not differing from the other endoderm cells, but marked off from those immediately external to them by not containing any of the brown matter. There is a single, central, cylindrical vascular bundle. In the basal leafless part of the aerial stem the scalariform tracheides usually form in transverse section an irregular incomplete ring or a series of groups circularly arranged, surrounded by and enclosing elongated elements with thin cellulose walls, some with long narrow nuclei, others without nuclei, apparently sieve-tubes. The central selerenchyma found in Psilotum is absent. In the leaf-bearing part of the stem the vessels occupy a central position surrounded by the phloëm elements. The vessels are smaller than in Lycopodium, the largest being little over 1 of an inch in diameter. In transverse sections of the leaf-bearing parts of the stem there is to be seen another tissue between the epidermis and the cortical layer, not forming a complete zone but arranged in fine masses; this is the mesophyll of the leaf-ridges, and does not differ from that of the leaves.

The leaves seem not to follow any definite law in their arrangement. In every $2\frac{1}{2}$ centimetres of the stem there are about five leaves, and of these the fifth is very usually directly over the first. About two centimetres below each leaf begins a longitudinal ridge of the stem, which, at first very low, becomes very prominent towards the base of the leaf. The leaves are situated with one edge directed inwards towards the stem, the midrib of the leaf meeting the stem at an angle of about 45° . The leaves are of a long oval shape, on an average a centimetre and a half in length and half a centimetre in breadth, having the base asymmetrically developed, the inner half of the lamina—that turned towards the stem—being more developed than the outer. The inner edge sometimes presents a few indistinct serrations, but in this there is much variation. There is a single unbranched midrib, which is produced at the extremity into a spine-like point.

The epidermal cells of the leaf have a lobed outline; their outer wall forms an irregularly thickened cuticle like that of the epidermis of the stem. Stomata may sometimes be found in equal abundance on both surfaces of the leaf, but in some cases they may be more abundant on one side than on the other, or they may be entirely absent on one side though abundant on the other. The mesophyll of the leaf and of the leaf-ridges consists of elongated cells which present at tolerably regular intervals short, blunt projections articulating with corresponding processes from neighbouring cells—a form of tissue resembling that found in the leaves of some species of Lycopodium, though in the latter the tissue is looser and the cell-processes more elongated.

Each sporangium ('sorus' in Bentham and Mueller's description) is borne on a special short side-branch which terminates in a symmetrical pair of leaves ('bracts') similar to the ordinary leaves, but smaller; the whole obviously representing the fertile cone-like

lateral branches of Lycopodium. The sporangium is situated on the side of this special branch which is turned towards the stem, immediately below the point where it gives origin to the two leaves. It has the form of two cones with their bases in apposition and their apices sometimes slightly bent upwards; the long axis lies parallel with the stem. Each cone is a loculus of the sporangium, the two cavities being separated by a delicate transverse septum. Along the ventral side runs a longitudinal suture—the line of dehiscence. When the sporangium dehisces the septum between the two loculi becomes ruptured, and the whole presents the appearance of being unilocular, and of having dehisced by two lateral valves. The wall of the sporangium consists of two layers —the epidermis, the cells of which are cuticularised, but not much thickened, and are elongated in a vertical direction, and a layer of small parenchymatous cells. The median septum contains a fine vascular bundle continuous with the central vascular bundle of the branch on which the sporangium is borne. The spores are 1 th of an inch in length; they are oval bodies, compressed, and with one side convex, the other concave.

The following is the description of *Psilotum* in the "Flora Australiensis":—

"Stems dichotomous, with distant notches bearing minute scalelike leaves, sometimes scarcely prominent, occasionally replaced by equally minute bifid bracts. Spore-cases usually three together, united in a capsule-like sorus, sessile in the axil of or attached to the bracts, nearly globular, 3-lobed, 3-celled, opening loculicidally in 3 valves. Spores minute, uniform."

And of the species—P. triquetrum, Swartz,

"Rhizome short, intricately branched. Stems erect, or pendulous when on trees, from 3 or 4 inches to about 1 foot long, repeatedly dichotomous in the upper part, the fertile branches 3-angled, the barren ones usually flattened. Scale-like leaves minute and subulate, the bracts subtending the spore-cases equally small and distant but forked. Capsule-like sori globular, about 1 line diameter, attached to the bract below the fork."

Psilotum triquetrum is much more widely distributed than Tmesipteris tannensis, being found in Asia, Africa, and America, as well as in the Australian colonies.

It differs considerably in general appearance from *Tmesipteris* owing to its shrub like habit, the absence of conspicuous leaves, and the repeated branching of the erect stems.

The rhizome is cylindrical and divides dichotomously. Its surface is finely striate so as to present a scaly appearance, and is covered with brown hairs. The single small central vascular bundle is of cylindrical form and consists of an inner bundle of scalariform vessels, an outer layer of phloem, with bast cells and sieve-tubes, enclosed in a sheath of short thin-walled parenchyma. Surrounding the sheath is a layer of brown matter similar to that observable in a corresponding situation in *Tmesipteris*, but less strongly developed. It seems to be arranged in longitudinal branching and anastomosing lines which are situated for the most part in intercellular spaces, out seem frequently to break into the cavities of cells. Outside of this is a thick zone of thin-walled parenchyma. The epidermal cells present no well marked cuticle. The aërial stems are marked by a series of longitudinal ridges; of these there are, as a general rule, five in any given section of the stem. These are connected with the leaves. The leaves are very small, narrow appendages, scarcely two millemetres in length, sparsely developed on the longitudinal ridges. In the case of the principal branches there are only single leaves; these are arranged with tolerable regularity, a leaf to about every two inches of each ridge. Where the leaf is inserted there is a notch in the ridge, the latter running on undiminished in size.

Each pair of leaves ('bracts') with the sporangium terminates one of the ridges. They are arranged with tolerable regularity, but there seems to be no definite phyllotaxial law. At the ends of the branches the leaves are closer together, and the growing point is surrounded by about three rudimentary leaves. The singular vascular bundle is nearly circular in transverse section, but the vessels themselves are arranged in five to eight groups more or

less completely united into a ring. Outside of the vessels is the phloëm which fills in the spaces between the groups of vessels so as to give the whole bundle a cylindrical form. The centre of the bundle is occupied by a strand of sclerenchyma. In the younger branches the sclerenchyma disappears, and the vessels are arranged in a flattened strand surrounded by phloëm. There is no brown matter, or only isolated spiral lines of it, and the endodermis is only distinguishable by its thinner cell-walls. The walls of the parenchyma cells are considerably thickened towards the periphery where their cavities are filled with chlorophyll granules. The epidermis has a very thick laminated cuticle. Stomata are abundant on the stem between the ridges, but there are none on the leaves nor on the ridges. The mesophyll of the leaves and of the leaf-ridges has the same peculiar form as in *Tmesinteris*: numerous short blunt processes from the walls of the cells articulating with corresponding processes from neighbouring cells, numerous anastomosing intercellular spaces being thus formed.

The wall of the sporangium has the same structure as in *Tmesipteris*, except that there are no vessels in the septa.

The spores, of which each sporangium contains a very large number, are of a rather narrow oval outline with a nearly straight ventral, and convex dorsal border. Along the ventral border runs a narrow line which marks the line of dehiscence of the two halves or valves into which the exospore splits to allow of the exit of its contents.

It is not to be wondered at, taking into account the want of success which has hitherto always followed attempts to cultivate the spores of *Lycopodium*, that in repeated experiments with the spores of both *Psilotum* and *Tmesipteris* under various conditions of substratum, light, heat, and moisture, I have hitherto failed to rear the prothallia of these genera.

9. On the Embryology of Vermilia cæspitosa and Eupomatus elegans.

In both species artificial impregnation is readily effected, and development proceeds with perfect regularity for at least two days in a glass vessel with an occasional change of water. After this period, however, abnormalities become frequent, and soon all the embryos become more or less deformed, until at the end of three or four days they all die off. To make the conditions as nearly as possible natural I reared the embryos in bottles, the mouths of which were closed with a piece of muslin; these were suspended by means of cords from the piles of a jetty in Port Jackson, or were attached to a buoy, the bottles being so placed as to be always immersed, but not far from the surface.

About half an hour after the contact of ova and spermatozoa yelk-division commences. Segmentation in *Vermilia* is equal and regular, as in *Serpula* and *Pomatoceros*. When four cells are formed the fifth and sixth are formed by the division of two of these, and the seventh and eighth are formed by division of the fifth and sixth.*

A central cavity soon forms itself in the morula, and at about the eighteenth to the twentieth hour invagination begins. A slight flattening appears on one side of the blastosphere, the side which is destined to become the posterior end of the embryo; a little on one side of this flattening a pit is formed, which growing inwards gives rise to the archenteron. The blastopore, at first nearly terminal, becomes shifted to one side of the larva, that side destined to become the ventral. At the same time it becomes elongated and slit-like, the anterior end of the slit widening to form

 $^{^*}$ In Psygmobranchus Salensky (Arch. de Biol. t. III. pp. 345-378) describes the segmentation as unequal.

the mouth, while in the neighbourhood of the posterior end of the slit the anus is formed at a somewhat later stage.*

When the process of invagination commences the larva is covered uniformly with cilia; soon, however, the anterior or cephalic end of the embryo loses its cilia, but becomes surrounded just in front of the mouth by a strong præ-oral ciliated band. The epiblast of the cephalic end becomes thinner than the rest except in the centre, where a thicker group of cells remains, destined to give rise to the cerebral ganglion.

The embryo becomes more elongated, assuming the shape of a pear, the broad end being the head and the narrow end the tail. From the centre of the former there grow out in most instances one, or sometimes two, long and slender motionless flagella; but these are frequently absent. The alimentary canal, though still simple in form, has now become densely ciliated internally, and undergoes frequent strong contractions. In sections made through an embryo at this stage, towards the end of the second day, a few, apparently irregularly placed, cells are to be found between the epiblast and the hypoblast; these probably form the foundations of the middle layer.

In the course of the third day the alimentary canal becomes differentiated into distinct esophageal, gastric, and intestinal regions. The præ-oral circlet of cilia becomes elevated on a distinct slightly oblique ridge, and a reniform eyespot becomes developed at a little distance from the ganglion, and connected with the latter by a fibrous strand. A thin-walled vesicle makes its appearance on the third day at the posterior extremity of the body, and soon attains a considerable size; it is apparently formed by involution of the epiblast, and remains connected with the exterior by a pore at the side of the anus.

^{*}Conn (Zool. Anzeiger, VII.) describes the blastopore in Serpula as becoming elongated and closed, the mouth and anus arising at its two ends. Salensky (l.c.) describes the mouth in Psygmobranchus as formed independently of the blastopore after the closure of the latter. In Spirorbis Götte ("Zur Entwick. der Wurmer" Zool. Anz. 1881, p. 189) states that the blastopore becomes converted into the mouth.

I have failed to followed the history of the embryo further by means of artificial cultivations, but have every reason to believe that I have found a later stage in the larval development of both genera in considerable numbers among the aggregations of their calcareous tubes. The form is precisely similar in both cases; but the larva of the Eupomatus, or what I take to be such, found among the Eupomatus tubes, is very much smaller, as one would be inclined a priori to expect, than that of the very much larger Vermilia. There is a broad head-lobe with two pairs of eyes, and, at the sides, tufts of strong cilia, which appear to be the remains of the pre-oral circlet. The body contains six segments, of which the first three are large and distinct, while the last three are smaller and not sharply marked off. The three anterior segments each bear a pair of bundles of very long and slender, slightly curved and minutely feathered provisional setæ, which the larva is in the habit of occasionally spreading out in the form of a fan; the last segment is provided with fasciculi of cilia. The buccal segment is amalgamated with the præ-oral lobe, and the mouth is a large aperture on the ventral aspect of the common segment thus formed. The alimentary canal is straight and simple, wide in front, narrowing behind towards the anus.